

THE TUBERCULIN TEST*

ITS VALUE IN THE DIAGNOSIS OF CHILDHOOD
TUBERCULOSIS—WITH A REVIEW OF
RECENT PROGRESS

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THE value of the tuberculin test in the diagnosis of childhood tuberculosis is based on the assumption that we are dealing with a specific reaction indicating infection. The certainty of this working hypothesis was challenged recently by Duken¹ and Baumler,² who tried to reexamine twenty-four of their patients from two to ten years later. These patients had had positive reactions to the intracutaneous test in the dilution of 1:1000 and had been negative to the Pirquet test in the first twenty-four months of life. It was impossible to trace four patients. Of the remaining twenty patients one died, and no postmortem examination was obtained. On reexamination fifteen patients were clinically and roentgenologically entirely negative and had a negative Mantoux by repeated tests up to two milligrams. The remaining four patients were infants, who had been tested during an attack of bronchopneumonia and who had subsequently died. They had either been repeatedly positive, or negative reactions had followed one positive reaction. Thorough post-mortem examinations including tissue sections on the four infants had failed to reveal tuberculous changes. The explanation may be that in the first two years of life the Mantoux test causes a traumatic inflammation of the skin tissue which lasts longer than forty-eight hours after the injection. The authors, therefore, regard the Mantoux test in the first two years of life as being convincing only if the less sensitive cutaneous or percutaneous test is positive at the same time.

NEGATIVE TESTS IN CHILDREN WITH
TUBERCULOUS INFECTIONS

The problem most frequently encountered is whether a negative test is conclusive proof of the absence of a tuberculous infection. The depressing influence on the reaction of various acute diseases is well known, especially measles, pneumonia, chicken-pox, epidemic meningitis, influenza, typhus abdominalis, and pertussis. The preallergic stage of a primary infection in tuberculosis is rarely seen. However, Happ and Casparis³ found the Pirquet test positive in 50 per cent of patients having miliary tuberculosis and tuberculous meningitis, and Engel⁴ found the Mantoux test positive in 94 per cent of these

cases, which means that the skin sensitivity is only depressed and by no means absent.

What is the probability of finding a negative skin test in patients who suffer from tuberculosis but do not show signs of an overwhelming infection?

Hetherington⁵ examined 2126 apparently healthy school children, eighty-two of whom had negative reactions to the intracutaneous injection of one milligram of tuberculin. Seven, or 8.5 per cent, of the nonreactors showed tuberculous nodules in the lung tissue and three, or 3.7 per cent, showed tuberculous tracheobronchial lymph nodes in the roentgenogram. There is a 20 to 1 probability that the tuberculin reaction will be positive in children who show lymph nodes in the roentgenogram, and a 100 to 1 probability in those revealing tuberculous tracheobronchial glands. On the other hand, children with latent lung infiltration, latent apical lesions and manifest tuberculosis had a positive skin test, without a single exception. In rare cases of keratitis phlyctenulosa (Harmstorf⁶) and of bone tuberculosis (Krause⁷), negative tuberculin reactions are observed.

RELATION OF ALLERGY TO IMMUNITY

The phenomenon of the disappearance of the positive test in children followed for many years leads to the question, what is the relation of allergy to immunity in tuberculosis? Various observers, for instance Austrian,⁸ found the Pirquet test negative after five years of observation of dispensary children, but did not apply the Mantoux test, in increasing concentration, to check the degree of desensitization. Krause maintains that allergy and immunity correspond in their manifestations. If a marked degree of allergy represents marked immunity, it is difficult to understand why a case reported by the same author showed complete disappearance of the sensitivity to tuberculin following the healing of the tuberculous process in bone and glands. As there is frequent need to make a differential diagnosis between active bone or joint tuberculosis and Legg-Perthe's disease, it is very consoling that a negative tuberculin test is absolutely conclusive against tuberculosis.

Rich,⁹ in opposition to Krause, states that "allergic hypersensitiveness is not necessary to immunity. Improvement appears in tuberculin therapy with the development of tolerance to tuberculin. Allergic reactions are proven to produce tissue necrosis." Desensitization would prevent this tendency, while leaving immunity intact. Allergy may be reduced to zero in the presence of progressive lung lesions. Moeller¹⁰ makes the interesting point that tuberculin used for testing is an abnormal metabolic preparation produced *in vitro*, and increase of the tuberculin reaction or desensibilization means the creation of artificial anaphylaxia or antianaphylaxia. Both processes have no direct relation to the natural immunity. The problem is still more complicated by the fact that the failure of the skin to react posi-

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tively is no proof of absence of allergy. Willis¹¹ inoculated guinea pigs with a low virulent strain of tubercle bacilli. The positive test disappeared slowly in the course of two years, but reappeared almost immediately after inoculation with a virulent strain of tubercle bacilli. In spite of a negative reaction, previous to the reinfection these pigs showed a high immunity compared with control animals. The conclusion may be drawn that reinfection is able to revive a vanishing tuberculo-allergy and immunity. These experiments and controversies have not only theoretical importance. In the evaluation of the B. C. G. immunization of infants against tuberculosis, the question is raised, has the vaccination protective value under the various degrees of allergy? Calmette,¹² on the basis of animal experiments, believes that even slightly positive or negative reactions after vaccination with B. C. G. do not disprove a rather high immunity. Wallgren¹³ and many others assume that immunity exists only with allergy. As the tuberculin test, even by subcutaneous or intracutaneous B. C. G. vaccination, becomes negative after one to one and a half years, frequent reinoculations are necessary to keep up the supposed stage of immunity. The whole issue seems to the observer to be far from clarified.

DEGREE OF THE TUBERCULIN TEST IN RELATION TO TUBERCULOUS PATHOLOGY AND SIGNS

The degree of the tuberculin reaction in relation to various factors and phases in childhood tuberculosis has found special consideration. In the children's chest clinic of the University of California Medical School 197 children reacted positively to the Mantoux test performed with one milligram tuberculin, and are graded as follows according to their infiltration:

Grade I. Infiltration 0.5 to 0.9 centimeter in diameter.

Grade II. Infiltration 1.0 to 1.4 centimeters in diameter.

Grade III. Infiltration 1.5 to 1.9 centimeters in diameter.

Grade IV. Infiltration 2.0 or more centimeters in diameter.

The following table shows the relation of the graded tuberculin tests to the percentage of positive x-ray findings:

This table shows that the percentage of positive findings increases as the grade of the tuberculin reaction increases.

The next consideration is the relation of the grade of the tuberculin reaction to the final diagnosis made on the patient.

| TABLE 1.—Positive Roentgenographic Findings | |
|---|---------------------------------|
| The Percentage of Cases in Each Grade Group | |
| Grade of Tuberculin Reaction | Percentage of Positive Findings |
| I | 18 |
| II | 25 |
| III | 34 |
| IV | 61 |

TABLE 2.—Grades of the Tuberculin Reaction in Relation to the Diagnosis of Specificity

| Grade of Tuberculin Reaction | PERCENTAGE OF CASES SHOWING | | |
|------------------------------|-----------------------------|---|--|
| | Active Tuberculosis | Any Evidence of Tuberculosis (Clinical or Roentgenological) | No Other Evidence of Tuberculosis Than Positive Reaction |
| I | 0 | 18 | 82 |
| II | 10.8 | 28 | 72 |
| III | 19.0 | 50 | 50 |
| IV | 22.6 | 69 | 31 |

No cases of active tuberculosis were found in patients showing a Grade I reaction, and only 18 per cent of these cases gave any evidence of tuberculosis. This is a marked contrast with those cases showing the Grade IV reaction—22.6 per cent of whom had active tuberculosis, and 69 per cent gave some evidence of the disease.

Opie¹⁴ has contributed an excellent study on the significance of the intensity of the tuberculin reaction in apparently healthy school children. His method differed from ours insofar as differing amounts of tuberculin from 0.01 to 1 milligram were injected and the intensity of the reaction recorded. He states that nearly all children with latent infiltration of the childhood type and three-fourths of the children with latent apical tuberculosis reacted to 0.01 milligram of tuberculin. Opie concludes "an intense tuberculin reaction may be associated with an apparently insignificant lesion, but the probability of grave infection increases with the intensity of the reaction."

METHODS OF TESTING

There are three questions regarding the choice of methods: Is the test accurate, easy to apply and innocuous? The intracutaneous and subcutaneous tests head the list in accuracy. Objections are raised against them because of the very marked local and systemic reactions which are occasionally encountered. We have used the dilution of 1:100 on ambulant children by the intracutaneous route for the past four years routinely and have had no disagreeable reactions other than an occasional vesicle formation, a slight temperature rise, or a moderately painful swelling of short duration. This routine procedure has the advantage that one injection will give definite information. Children in the hospital with symptoms or signs of activity should be tested with smaller doses at first, especially children with lung tuberculosis, as I have seen hemorrhage following the intracutaneous injection of one milligram of tuberculin.

The Pirquet or cutaneous test is easier to apply and causes much less local tissue reaction, but has an error estimated to be 15.8 per cent (Reiss¹⁵), 55 per cent (Smith¹⁶), and 58 per cent (Hille¹⁷).

ADVANTAGES OF PERCUTANEOUS TEST

The necessity of applying an instrument puts the Pirquet test and the Craig test (Forbes¹⁸), with its multiple puncture method, at a disadvan-

tage when compared with the so-called percutaneous methods of testing. The percutaneous test, performed by rubbing tuberculin ointment into the skin, is most popular in Europe for testing school children on a large scale. Various preparations containing concentrated tuberculin, with and without the addition of killed bacilli, are used. The most popular preparations are Ektebin Moro and Dermotubin Loewenstein. The procedure is simple. Reactions are read after forty-eight hours, or even up to one week later, and consist in the appearance of inflamed hair follicles over the area of testing. A weak or miliary medium, or a confluent and strong or vesicular reaction, are easily distinguished and compare roughly with the 1-3 plus reactions of the Mantoux test. The error is estimated to be 5.2 per cent (Harms and Seitz¹⁹) in ambulant infected children, 9 per cent in active tuberculous hospital patients, and 22 per cent in latent tuberculous hospital patients. We used the ointment test (dermotubin) on more than forty ambulant children and had only one discrepancy of reaction as compared with the intracutaneous injection of one milligram of tuberculin.

The Pirquet test introduces 0.01 milligram of tuberculin into the skin as estimated by Happ and Casparis.³ All latent and active chest lesions in ambulant children react to 0.01 mg. of tuberculin, as found by Opie.¹⁴ Therefore it may reasonably be assumed that the ointment test, which gives far less errors than the Pirquet test, is accurate enough for school examinations. In Vienna over forty thousand children entering the first grade have been tested since 1926 by this method to which the parents, with very few exceptions, did not object.

CONCLUSIONS

1. The intracutaneous tuberculin test is specific with the exception of children up to twenty-four months, where nonspecific reactions are observed in rare cases.

2. The tuberculin test is sometimes negative in apparently healthy children with nodules or calcified tracheobronchial glands in the roentgenograms.

3. The exact relation of allergy to immunity in childhood tuberculosis is unknown.

4. The intensity of the tuberculin reaction increases in proportion with the percentage of children:

- (a) Having roentgenographic chest findings.
- (b) Having active tuberculosis.
- (c) Having clinical and roentgenographic evidence of tuberculosis.

5. The intracutaneous tuberculin test represents the most satisfactory method for hospitals and dispensaries, and the percutaneous or tuberculin ointment test is the most convenient method for institutions, schools, and private practice.

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DISCUSSION

C. F. GELSTON, M. D. (384 Post Street, San Francisco).—Of the greatest value in investigating bronchial adenopathy in children is the correlation between clinical and roentgenological findings. Interpretation and diagnosis have been much hindered through the absence of such data. Of great aid, further, are clinical researches such as are here presented. In the first place, it is extremely significant that the intensity of the reaction increases directly with the roentgenological and clinical findings. This has often been doubted. Secondly, the occasional negative reaction in what appears roentgenologically to be tuberculous is of vital interest since it is just here that differentiation from tuberculous and nontuberculous infection is so important. The data obtained so far in this study are so informative that they will unquestionably stimulate further enlightening research.

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LLOYD B. DICKEY, M. D. (Stanford University Medical School, San Francisco).—In any two diagnostic tests of equal accuracy, that performed with the least instrumentation will, of course, be the most practical. One must be careful not to sacrifice accuracy merely to prevent a slight and transient discomfort to a patient. In our clinic at Stanford University Medical School we have had no opportunity to use the ointments, but have compared the Craig and Mantoux tests. In the Craig test the instrumentation is practically negligible, and in about a hundred cases we have found it to be fully as accurate as the slightly more painfully administered Mantoux. We long ago dis-

continued the use of the Pirquet test, as it is obviously so inaccurate as to be impractical for clinic practice. If further studies prove the tuberculin ointment tests to be as accurate as the intracutaneous method, it will probably replace all those now in use.

In a study of the degree of skin sensitiveness to tuberculin, as administered intracutaneously, certain groups of our clinic children reacted more strongly than the average. Giving significantly stronger reactions were those suffering from bone and joint tuberculosis and phlyctenular conjunctivitis, children with recent contact to open tuberculosis, Chinese and Japanese children. In the contact children, and in the Orientals, this suggests that reinfection heightens the allergy in tuberculosis.

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OSCAR REISS, M. D. (1930 Wilshire Boulevard, Los Angeles).—Doctor Wolff's very interesting paper is a most stimulating one and leads us to wonder whether we do not have to reevaluate some of our old ideas regarding tuberculin skin tests in children.

In regard to the negative Mantoux reaction one may still believe that except in the case of certain well known and well recognized exceptions, provided that a potent tuberculin has been used, it signifies the absence of tuberculous infection.

To be able to interpret a positive Mantoux in terms of degree of involvement, as proven by x-ray and clinical evidence, certainly widens materially the usefulness of tuberculin tests. Doctor Wolff's classification is worthy of wide adoption.

My own experience with the percutaneous test has been very limited, but I believe it offers the most desirable type of test and merits an extensive trial. For the past twelve or thirteen years I have depended entirely on the intracutaneous method of Mantoux using initially .01 milligram increased to .1 milligram and 1 milligram if necessary. I have on two occasions carefully checked my results in a considerable number of cases with a von Pirquet done simultaneously, and have found the Mantoux far more reliable. I hope in the near future to check a series of cases with the percutaneous method.

THE LURE OF MEDICAL HISTORY*

ESSAYS ON THE HISTORY OF EMBRYOLOGY†

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IX

SPALLANZANI

It is easy to understand Bonnet's enthusiasm over Spallanzani's experiments on cross-fertilization as expressed in the following words from his letter of January 13, 1871:

"You are now in possession of a sure and easy way of ascertaining what species can procreate together; and the experiments you propose attempting next spring, by putting your voluptuous spaniel in the company of cats and rabbits, promise not so fair as those which you will make, by introducing the semen of this spaniel into the uterus of a doe-rabbit and a she-

cat, and on the other hand, by introducing the semen of the male rabbit and cat into the uterus of a bitch. You hold in your hand a precious clue, which will guide you to the most important and unexpected discoveries. I know not, whether what you have now discovered, may not one day be applied in the human species to purpose we little think of, and of which the consequences will not be trivial. You conceive my meaning: however that may be, I consider the mystery of fecundation as nearly cleared up."

Bonnet surmised that,

"From your numerous experiments on artificial fecundation it appears that attempts of this kind upon germs, while yet in the ovium, or at the upper end of the ducts, will fail. I can, I think, assign the reason. The seed acts on these fetuses as a simple stimulant. Now there is an original relation between the latent power which causes the irritability of the contraction of the muscular fibre. If it has not yet attained the necessary degree of consistence, it will not be in proportion to the mode of action of that power, and its impression will therefore produce no effect. The germ must have arrived at a determinate growth before it can be susceptible of irritation. Such was the reasoning of the great Haller."

Although Spallanzani was a preformationist and believed that the amnion and umbilical cord exist in an invisible form, before fecundation, his investigations on fertilization rank very high indeed. They are outstanding for their number and ingenuity. The modest title of the two volumes, the one on plants and the other on animals, give little intimation of the many splendid experiments recorded there. In the introduction Spallanzani says that the subject of his second dissertation is "Artificial Fertilization," the first outlines of which may be seen in his "Prospectus Concerning Animal Reproduction." By artificial fecundation Spallanzani means insemination, of course. He says, "This was accomplished by means of the seminal liquor of the animals themselves; and I have succeeded as well as if the male himself had performed his proper function."

It is strange that by his experiments on plants, Spallanzani was convinced that the "fecundating dust," or farina foecundans as it was called, that is pollen, was "not so absolutely necessary as botanists in general suppose." However, he had an entirely correct attitude toward the problem of fertilization for he added, "It is said by many that fecundation is among the mysteries of nature; and like many of her operations, an object of admiration, rather than of inquiry. Such an opinion is highly agreeable to the idleness of man." He repeated the experiment of Swammerdam and Roesel von Rosenhof and found that mating frogs will discharge their sexual products even when confined, thus contradicting the statements of Linnaeus and Vallisneri in this regard. Vallisneri, it seems, held that the female frog will not lay eggs when separated from the male, but Spallanzani showed that she will do so if the separation is effected after the eggs have descended into the cavity of the uterus (oviduct), but that they will be sterile. Since it is now well known that the female frog will not lay eggs if wholly unmated, Vallisneri was right. The frogs Spallanzani used must have mated long enough for ova to descend into the oviducts, when expulsion instead of absorption will take place. Since

* A Twenty-five Years Ago column, made up of excerpts from the official journal of the California Medical Association of twenty-five years ago, is printed in each issue of California and Western Medicine. The column is one of the regular features of the Miscellany Department of California and Western Medicine, and its page number will be found on the front cover index.

† This is the ninth paper of a series of essays on this subject. Previous papers were printed in this journal as follows: Part I, in December California and Western Medicine, page 447; Part II, in January number, page 40; Part III, in February number, page 105; Part IV, in March number, page 176; Part V, in April number, page 241; Part VI, in May number, page 341; Part VII, in June number, page 394; Part VIII, in July number, page 41.